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How to Catch a Small Beetle: Top Tips for Visually Screening Honey Bee Colonies for Small Hive Beetles

Bram (A.C.M.) Cornelissen  and Peter Neumann

Introduction

As of 2017, the small hive beetle (SHB, *Aethina tumida*, Murray; Coleoptera: Nitidulidae) can be found on all continents except Antarctica (Neumann, Pettis, & Schäfer, 2016; Schäfer et al., 2018). Earmarked an invasive alien species, it can rapidly become established in regions outside its natural range probably also because it can decimate colonies of European honey bee, *Apis mellifera*, subspecies in a short span of time (Neumann et al., 2016). In order to anticipate an introduction of the SHB to new areas, early detection by monitoring is key. Only when SHBs are detected before reproduction occurs can eradication succeed (Schäfer et al., 2018). However, even if SHBs become established, they can be contained when conditions (geography, climate, etc.) are favourable. When the SHB was first spotted in Italy in 2014, it was the larvae that gave it away (Mutinelli et al., 2014). In recent years, SHBs have been found in local feral colonies in Italy. Even though SHB is established in the region, containment efforts appear to be successful, thereby limiting its spread.

Currently, the most used strategy for monitoring invasion of SHB consists of using sentinel colonies (three to five frames of bees) as trap hives (Schäfer et al., 2018). These hives are placed around high risk areas (e.g., sea ports) and checked regularly for the presence of SHBs (see Neumann et al., 2016, for a list of countries implementing this strategy). Additionally, in-hive SHB traps can be used to make capturing SHBs easier. Visual screening of colonies is currently considered the most effective diagnostic method for SHB, specifically when looking for low numbers of individuals in an early stage of invasion

(Mutinelli et al., 2014; Neumann et al., 2013). Furthermore, this can be applied by various stakeholders (e.g., beekeepers, veterinarians) as well and included in monitoring strategies. Visual screening for SHB does, however, require knowledge of beetle behaviour. Here we provide tips for screening colonies for SHBs. First, the focus is on beetle behaviour and appearance (1–4), followed by notes to the observer (5–9).

Tip 1: Beetles do what beetles do

SHBs tend to prefer the perimeter of the bee nest, including the outer frames, walls, bottom and underneath the lid of hive (with up to 50% being found on the bottom boards; Neumann et al., 2016; Spiewok et al., 2007), though they can be found anywhere in a hive (Lundie, 1940). Knowing how SHBs react to hive manipulation can help one anticipate their next move and, as such, increases the likelihood of catching them. SHBs exhibit a range of behaviours to cope with their honey bee hosts and possible predators, to avoid detection and capture (Ellis, 2005; Ellis & Hepburn, 2006; Neumann et al., 2016). These behaviours are also shown by SHBs when a hive is being inspected. In short, these are: run, hide, drop from the comb and fly.

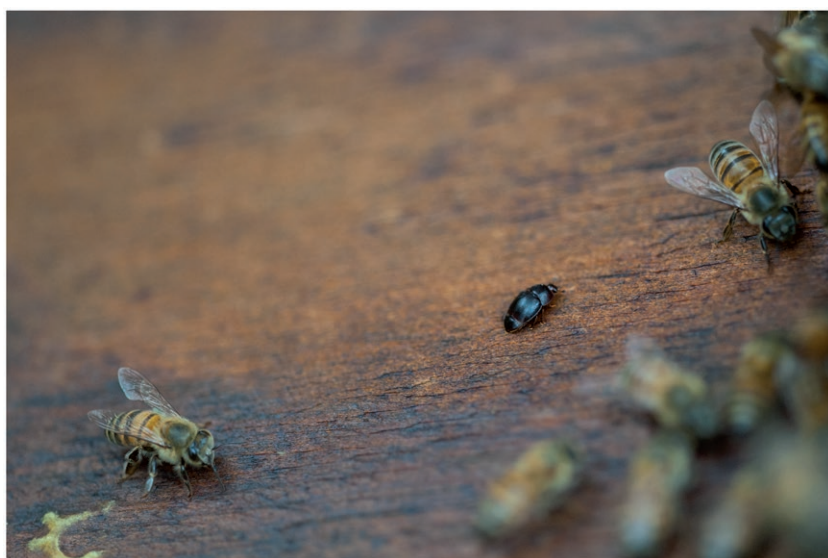
Typically, SHBs run away from perceived threats. Although more classifications of walking movement (walk, run, flee) are given by Neumann et al. (2016), we will refer to this only as running herein. Although they have the opportunity to fly at any time, field experience tells us that they are much more likely to run from threats. In doing so, they seek shelter from light and from bees trying to catch them (Figure 1). Often, SHBs follow edges and corners when running, but when forced

to, they will also run into the open. If a SHB is seen running on a frame during inspection, it will usually try to get to the darker, shady side of the frame. All subspecies of *A. mellifera* corral SHBs in constructed prisons as part of social encapsulation (Ellis, 2005; Neumann et al., 2001). These prisons are often found in between the lid and top bars of a hive. Thus, removing the lid can initiate a SHB prison break. That is when SHB propensity to hide comes into play. SHBs can hide in empty brood cells, cracks and crevices of hive parts and frames, but also under the cover of bees (Schmolke, 1974). If already in hiding, they are sometimes difficult to coerce into the open. However, SHBs often leave less-than-optimum hiding places in search of better ones, thus revealing their location. For instance, we have often seen SHBs running over a brood comb, seeking shelter in empty brood cells, running from one to the other (Figure 2), to escape being captured. Even so, hiding SHBs are notoriously difficult to spot, even if covered by a few bees (Figure 3). A thorough investigation of all hive parts is required to increase the chance of an accurate diagnosis (Neumann et al., 2013; see tip nr9).

SHBs can also “drop” from the surface where they are found when they feel threatened. This behaviour can be overlooked. For instance, when a lid is removed from a hive, SHBs hiding there can react instantly by falling from the lid, this even before the lid is flipped for inspection. Falling SHBs either end up somewhere half-way down in the hive or they drop further to the bottom of the hive. Other circumstances that provoke this behaviour occur when SHBs are persecuted in the process of being captured. When running from a threat is no longer an option, they will retract their legs and drop.



■ **Figure 1.** Adult SHBs running over a honey bee brood comb (*A. mellifera scutellata*) and hiding in empty brood cells. Please click the image to play Video 1. Alternatively, this video can be viewed via the supplemental material tab here: <http://dx.doi.org/10.1080/0005772X.2018.1465374>. Video recorded in the laboratory of SIRG at the University of Pretoria, South Africa, December 2015. Photo & video credit: A.C.M. Cornelissen.



■ **Figure 2.** SHB seen running and trying to hide from honey bees (*A. mellifera scutellata*) on a flipped over lid of a hive at the experimental farm of the University of Pretoria, November 2015. Please click the image to play Video 2. Alternatively, this video can be viewed via the supplemental material tab here: <http://dx.doi.org/10.1080/0005772X.2018.1465374>. Photo & video credit: A.C.M. Cornelissen.

SHBs are also excellent flyers (Lundie, 1940; Neumann, Hoffmann, Duncan, Spooner-Hart, & Pettis, 2012). Once on the wing, they can be out of sight in seconds. Escape by flight is probably more common than it is actually observed, simply because flying SHBs are difficult to spot. Before flight commences, SHBs pause their movement for less than a second, at which time they expand their elytra and unfold their wings. Some beetle species seek out high grounds before lift-off (Johnson, 1969), SHB does not. Once this behaviour is known to the observer, SHB flight can be anticipated. If lucky,

SHB flight can be prevented by a swift and accurate downward countermovement with the hand. However, SHBs often try repeatedly to fly away, so containing the SHB instantly and adequately is advised (e.g., in a box or using an aspirator).

Tip 2: Tough egg to crack

Mated SHB females can start ovipositing almost immediately after finding a suitable food source (De Guzman, Rinderer & Frake, 2015), thus keeping an eye out for eggs and larvae is important. However, SHB reproduction does not



■ **Figure 3.** Flipping the lid, reveals a couple of SHBs, clustering underneath a group of bees. Photo credit: A.C.M. Cornelissen.

constantly happen and mostly occurs at cryptic low levels (Spiewok & Neumann, 2006). In such cases, finding SHB eggs or larvae is unlikely. When SHB mass reproduction occurs (Neumann & Elzen, 2004), the signs are very obvious, with many larvae present (tip 3), often combined with a typical smell and slime (tip 4) (Ellis, 2012).

SHB eggs, though, are usually hard to find, due to the indistinct places they are laid and their small size (~1 mm). Actively searching for eggs is, therefore, not a realistic option. One indicator, though, is the presence of punctured sealed brood cells, as females occasionally lay eggs in brood (Ellis, Hepburn, Delaplane, & Elzen, 2003). However, other factors can explain such deformities including *Varroa destructor* (or detection thereof), foulbrood or hunger (Spivak & Gilliam, 1998). Checking for the actual presence of SHB eggs in those cases is required. Eggs are typically oviposited in clusters of various sizes (Figure 4, Lundie, 1940). If other life stages of the SHB are absent, confirmation by molecular diagnosis (Ouassou Idrissou, Huang, Yañez, Akinwande, & Neumann, 2018) is essential to confirm the species and to distinguish the eggs from those of other hive dwellers (e.g., *Braula* spp.).

Tip 3: Larvae all around

At cryptic low-level reproduction, few SHB larvae might occasionally be observed in hive debris accumulated on the bottom board. Like adults, immature SHB larvae will hide when exposed to light (Neumann et al., 2016), so sifting through hive debris will set them in motion (Schmolke, 1974). If mass reproduction occurs, SHB larvae are more likely to be found on combs carrying a source of food (bee brood is preferred, honey or pollen; Ellis, Neumann, Hepburn, & Elzen, 2002). Taking a frame from a hive will make them crawl out and drop. At first sight wax moth – larvae look



■ **Figure 4.** Small hive beetle egg clusters oviposited between two microscope slides spaced apart approx. 0.3 mm. Photo credit: A.C.M. Cornelissen.

similar in shape and size (Ellis, Graham, & Mortensen, 2013) though these are generally less active. Since very similar looking beetle larvae may also be found in hives (Maitip et al., 2017), identification by a taxonomist or PCR (Ouessou Idrissou et al., 2018) is required for positive identification of SHB larvae.

Tip 4: A yeasty business

In coherence with mass reproduction, smell and slime are important indicators for post hoc (after colony collapse) SHB diagnosis (Ellis, 2012). The SHB often carries the yeast *Kodamaea ohmeri*, which under favourable condition flourishes in SHB-infected bee hives (Benda, Boucias, Torto, & Teal, 2008). A pungent acidic smell of fermentation, similar to wild fermented beers (the yeast is used for fermenting pickles) can be observed in combination with a slimy glistening coating. This coating mantles the combs (Figure 5). Hive products become fluid due to the accumulation of water. When a surplus of honey is present, the resulting slime can be found in and around the colony. It must be noted, however, that the slime is not always associated with mass reproduction. As soon as honey is lacking in the larval food, the reproduction becomes “dry” and the smell as well as slime are not present (Neumann & Härtel, 2004).

Tip 5: It takes two

Screening colonies for SHBs requires at least two individuals. A preferred task division would be one person manipulating the hive parts and a second one capturing SHBs. Both, however, should be visually screening the same area simultaneously if possible.

Tip 6: Knock, shake, blow

Do not trust your eyes. Once a hive is opened, it is essential to use all means necessary to get SHBs to leave their hiding



■ **Figure 5.** The signs of SHB mass reproduction: larvae and slime. Drone brood comb, heavily infected with SHB larvae of different developmental stages. This brood was collected for research purposes and incubated in the laboratory at 30°C. It contained an adult female SHB, resulting in mass reproduction. Please click the image to play Video 3. Alternatively, this video can be viewed via the supplemental material tab here: <http://dx.doi.org/10.1080/0005772X.2018.1465374>. Video recorded in the laboratory of SIRG at the University of Pretoria, South Africa, November 2015. Photo credit: A.C.M. Cornelissen.

spots. As mentioned, bees should be shaken off the frames and hive parts and sifted through with a hive tool or hands. All frames and hive parts should be knocked on the edges on a hard surface (Neumann et al., 2013). SHBs hiding in cells will drop. Blowing in corners and cracks can also help as the flow of air can initiate SHB movement.

Tip 7: Let there be light

On the one hand, bright sunny conditions can increase the chance of SHBs moving out of bounds. On the other, a light source can be a useful tool in spotting the dark-coloured adult SHBs in dark corners and thus forcing them from where they are hiding. In some countries, black plastic foundation is used in frames. Finding hiding SHBs will be much more difficult when foundation such as this is used. Therefore, we advise using bright-coloured hive material as much as possible. For instance, new wooden frames and year-old drawn comb instead of older combs increase the chance of spotting SHBs.

Tip 8: Trap them if you can

There are many types of in-hive traps available for catching SHBs, most of which are meant to control SHB. For diagnostic purposes, strips of corrugated plastic are recommended (Schäfer, Pettis, Ritter, & Neumann, 2008, 2010). The strips should

be inserted through the hive entrance and left for 48 hours. Collecting SHBs should be done swiftly, by pulling the strip out and knocking the content in a container. Equipping a hive with a diagnostic strip can make visual screening more efficient as on average 30% of the SHB in a colony can be captured in that way and in established populations a detection rate of 96.3% can be obtained (Schäfer et al., 2010). How these strips perform in the early stages of SHB invasion, when low numbers of individuals are present, has not been investigated. The first response to the outbreak in Italy showed that traps should only be used as an additional tool to visual screening as the results were poor compared to earlier studies (Mutinelli et al., 2014; Schäfer et al., 2010).

Tip 9: Lid first, a standard approach

It is crucial to apply a systematic approach when screening a hive for SHBs (Neumann et al., 2013). Prepare by placing a hive on a white surface covering at least 200% of the total surface area of the hive. This will prevent SHB escapees leaving the hive from going unnoticed. Also, set up two empty clean hive boxes, in which the inspected frames can be placed and one in which the frame carrying the queen can be placed (Figure 6). Make sure the boxes have as few cracks as possible. For example, use a sealing-kit to line the edges and corners. Other preparations should include having



Figure 6. A set up for screening nucs for SHB adults using two empty boxes. Although this set up works well in general, there is no white surface underneath the nucleus colony, allowing SHBs to escape unnoticed. Photo credit: A.C.M. Cornelissen.

an aspirator ready as well as a second white surface to shake bees onto. Also prepare a hard surface (wooden) to knock frames and other hive material onto.

In short a best practice procedure should include the following:

- Remove the hive lid and screen it and the top bars for SHBs. Aspirate fleeing SHBs.
- Find the frame with the queen and place it in a separate box. Afterwards, inspect all the remaining frames for SHBs. Carefully and systematically screen the frames from top left to right bottom. Flip the frame and check the other side.
- Place all screened frames in the second clean box.
- Inspect the now empty original hive body. Check the sides, remove the box if possible and check the bottom. Flip it over to check underneath. Shake all bees present on the hive parts onto a white sheet and sift through for SHBs. Check the hive components again.
- Now, one-by-one inspect the frames, starting with the ones on which the queen is not found. Inspect the frames. Shake off the bees. Sift through the bees and check the empty frames. Knock the empty frames on a hard white surface by holding the lower corners and tapping the top bar onto the surface. If filled with unripe honey, skip the knocking. Shake the bees back into the box and return the frames.
- Return the queen back to the hive before inspecting the frame she was on.

Final Remarks

No screening method is beetle-proof. Studies have shown that visual screening

combined with in hive traps can be used effectively for SHB diagnosis of honey bee colonies (Neumann et al., 2016). It is important to remember that the untrained eye will be less likely to identify SHBs and also may lack the skills to anticipate its behaviour. We recommend gaining experience with SHB field diagnostics.

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